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Elucidation of the transport mechanism of baicalin and the influence of a *Radix Angelicae Dahuricae* extract on the absorption of baicalin in a Caco-2 cell monolayer model

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ABSTRACT

Ethnopharmacological relevance: *Angelicae Dahurica (Hoffm.) Benth. & Hook. f. ex Franch. & Sav* combined with *Radix Scutellariae baicalensis Georgi* has been widely used in traditional Chinese medicine (TCM) as an antipyretic analgesic and anti-inflammatory drug. Modern pharmacological studies have demonstrated that the compatible application of these two drugs is an effective treatment for hepatitis. A previous study indicated that a *Radix Angelicae Dahuricae* extract enhanced the intestinal absorption of the baicalin found in *Radix Scutellariae*; however, the underlying compatibility mechanism of these two herbs remains unknown. In this study, we further examined the effect of a *Radix Angelicae Dahuricae* extract on the absorption and transport properties of baicalin in a Caco-2 cell model to determine the compatibility mechanism of these two herbs.

Aim of the study: The aim of this work was to study the transport properties of baicalin in *Radix Scutellariae* across cell membranes and the effects of a *Radix Angelicae Dahuricae* extract on baicalin absorption using the well-characterized, human-based intestinal Caco-2 cell model.

Materials and methods: We assessed the absorption, bidirectional transport and toxicity of baicalin using a range of parameters, including drug concentration, pH, a P-glycoprotein (P-gp) inhibitor (Verapamil), an MRP inhibitor (MK-571) and EDTA-Na_2 (tight junction modulator). Next, we studied the influence of a *Radix Angelicae Dahuricae* extract on the transport of baicalin under the same conditions. Drug concentration was measured by HPLC, and the apparent permeability coefficient (Papp) and apparent permeability ratio (PDR) were subsequently calculated.

Results: The results showed that baicalin is non-toxic within a concentration range of 800 μg/mL to 4800 μg/mL. The transport of baicalin showed time and concentration dependence. The absorption of baicalin was optimal at pH 7.4 in 37 °C; however, the absorption decreased at 4 °C. The Papp of baicalin transport through the Caco-2 cell monolayer model was altered when specific inhibitors of P-gp or MRP were added to the cells. However, there was no significant difference in the PDR value. The Papp of baicalin improved when it was combined with the *Radix Angelicae Dahuricae* extract. The influence of EDTA-Na_2 on the transport of baicalin showed that the permeability of baicalin significantly increased. The result further indicated that the mechanism of baicalin intestinal absorption in the Caco-2 cell monolayer involves passive transcellular diffusion.

Conclusions: Passive diffusion is the main mode of intestinal absorption of bacalin and it involved in the efflux of proteins. The enhanced intestinal absorption of baicalin by *Radix Angelicae Dahuricae* can be due to opening of the tight junctions between cells and inhibition of MRP efflux protein expression or function.

1. Introduction

Baicalin (Fig. 1) is the main active flavone extracted from *Radix Scutellariaee*, a plant used in traditional Chinese medicine. This compound is also the primary quality indicator used to evaluate *Radix Scutellariaee* preparations. Baicalin has been shown to have anti-oxidative (Guo et al., 2011), anti-bacterial (Hu and Feng, 2001), anti-viral (Xu et al., 2007), anti-carcinogenic (Tang et al., 2007) and anti-allergic (Gao et al., 1998) properties; furthermore, studies have shown that baicalin has anti-inflammatory, anti-thrombotic, vasodilator and antipyretic effects in multiple peripheral tissues and organs (Cheng et al., 2006; Li and Ge, 2010). Similar to other flavones, baicalin has low water solubility and

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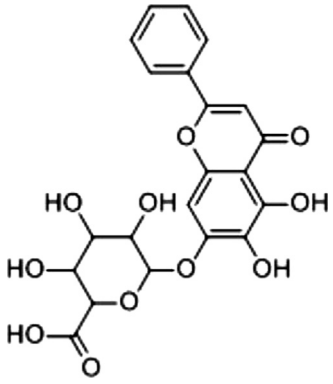

Fig. 1. Chemical structure of baicalin.

permeability that limits its absorption and bioavailability (Liu et al., 2006; Che et al., 2001). In general, the intestinal absorption barrier is a major factor that limits the absorption and oral bioavailability of drugs. Therefore, research into novel methods and the compatibility of additional compounds that can overcome the barrier of intestinal absorption are needed to study the intestinal transport mechanism of a drug and to increase its bioavailability.

Aherb-pair of traditional Chinese medicine (TCM) is a relatively fixed composition of two herbs used in a clinical treatment and represents the most basic form of compatibility application in TCM. Clinical experience and modern research has shown that the application compatibility of herb-pairs in TCM can reduce toxicity, increase drug activity and improve the pharmacological effects of the drug (Xi and Chen, 2008). Radix Scutellariae and Radix Angelicae Dahuricae are a common herb-pair (Xu, 1996). The main components of Radix Angelicae Dahuricae are coumarin and volatile oil. It has been reported that volatile oil can promote drug absorption and coumarin can inhibit the activity of metabolic enzymes (Li et al., 2007; Li et al., 2006). However, little is known about how Radix Angelicae Dahuricae influences the absorption of baicalin.

Caco-2 cells are derived from a human colorectal carcinoma and is a widely used cell line (Pinto et al., 1983). Permeability across the fully differentiated Caco-2 monolayer is considered to be a model of intestinal absorption (Hilgers et al., 1990). Indeed, the Caco-2 cell system is an in vitro absorption model that is routinely used in both industry and academia to study intestinal permeability. These cells also express nutrient and drug transporters that allow for the study of carrier mediated uptake and efflux mechanisms (Hayeshi et al., 2008). Here, we chose to utilize the Caco-2 cell monolayer model to study the absorption of baicalin.

Based on the use of the Caco-2 cell model, a P-gp inhibitor (Verapamil) and MRP inhibitor (MK-571) were chosen to investigate if the transport of baicalin was affected by two drug intestinal cellular efflux transporters (P-gp and MRP). It is well-known that the application compatibility of medicines can change the transport process of drugs. Moreover, *Radix Scutellariae* and *Radix Angelicae Dahuricae* are frequently combined for use in traditional Chinese medicine. In this study, we studied the possible mechanism behind the enhancement of baicalin absorption with a *Radix Angelicae Dahuricae* extract. These data can further explain the compatibility of the *Radix Angelicae Dahuricae* and *Radix Scutellariae* herb-pair.

2. Materials and methods

2.1. Reagents and chemicals

Radix Angelica Dahuricae and *Radix Scutellariae* were purchased from Bozhou Baixin Pharmaceutical Co., Ltd. (Anhui, China) and both the botanical identifications were authenticated by Professor Shouwen Zhang. The voucher specimens of *Radix Angelicae Dahuricae* (No. 074401) and *Radix Scutellariae* (No. 113323) were deposited in the Herbarium of the Department of Pharmacognosy at Jiang Xi University of Traditional Chinese Medicine.

Baicalin, with a batch number of 101765–201108, was bought from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, China). The water used in this study was purified using a Milli-Q water system (Millipore, Bedford, MA, USA). HPLC grade solvents from Merck (Germany) were used for drug analysis.

Fetal calf serum (FCS) and Dulbecco's modified eagle's medium (DMEM) were purchased from Hyclone (Thermo Fisher Scientific). Penicillin and Streptomycin solutions (10,000 U/mL Penicillin and 10,000 μg/mL Streptomycin) and Hank's Balanced Salts Solution (HBSS) were purchased from Solarbio (Beijing Solarbio Science & Technology Co., Ltd., China). Non-essential amino acids were obtained from Sigma Chemical Co. (USA). Trypsin-EDTA solution (0.25% (w/w) trypsin/1 mM EDTA) was supplied from Gibco Laboratories (Life Technologies Inc., USA). Verapamil hydrochloride and MK-571 were purchased from Sigma Chemical Co. (USA). Twenty-four-well cell culture plates were purchased from Costar (Corning Incorporated, USA). Millicell® Cell Culture inserts and Millipore Express® PES Membranes (0.22 μm) were obtained from Millipore (USA).

2.2. Cell cultures

The Human colon adenocarcinoma cell line Caco-2 was obtained from the American Type Culture Collection (ATCC, USA) and was maintained at +37 °C in an atmosphere containing 5% CO_2 at 95% relative humidity. Caco-2 cells were cultured in medium containing DMEM (high D-glucose 4.5 g/L), 10% fetal bovine serum (inactivation at 56 °C for 30 min), 1% non-essential amino acids, 1% L-glutamine, and a penicillin–streptomycin double antibody solution. The medium was changed every other day during cell growth and differentiation. The cells were passaged at a 1:3 ratio every four days when they had grown to 80%–90% confluence. The cells were harvested from plastic flasks (75 cm^2) with 0.25% EDTA-trypsin. For transport experiments, the cells were seeded onto polycarbonate filter membranes (pore size 0.4 μm, filter area 0.6 cm^2) in 24-well plates at a density of 1×10^5 cells/cm^2 and grown for 21 days before the transport experiments. Using a previously described method (Sun et al., 2006), transepithelial electrical resistance (TEER) and alkaline phosphatase activity were measured. The Millicell membranes with monolayer cells that met defined criteria with a TEER of above 500 Ω/cm^2 were used for the transport studies. Before the experiment, the monolayer cells were washed twice with 37 °C blank Hank's buffered salt solution (HBSS solution, pH 7.4) and then placed in a 5% CO_2, 37 °C incubator for 30 min.

2.3. HPLC of baicalin

An Agilent 1260 series HPLC system (Agilent Technologies, USA) comprised of a G1311A quaternary pump module, a G1316A-colcom column oven and chiller, a G1314 AVWD detector coupled with an analytical workstation, an on-line G1379B-infinity degasser, and a G1313A-autosampler was used. Reversed-phase chromatography was applied using an analytical Phenomenex Luna5uC18 column (250 × 4.6 mm, 5 μm). The mobile phase consisted of A (acetonitrile)/B (0.2% phosphoric acid) (24/76) with a 1.0 mL/min flow rate, and the detection wavelength was set at 250 nm. The column temperature was 25 °C and the injection volume was 20 μL.